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UPTAKE AND UTILIZATION OF DISSOLVED GLYCINE BY *AURELIA AURITA* SCYPHISTOMAE: TEMPERATURE EFFECTS ON THE UPTAKE PROCESS; NUTRITIONAL ROLE OF DISSOLVED AMINO ACIDS

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During the last decade there has been a considerable renewal of interest in the uptake of dissolved organic matter (especially amino acids) by marine invertebrates. Among the goals of these investigations is the elucidation of the importance of dissolved free amino acids to the nutrition of these animals (for recent reviews and bibliographies, see: Johannes, Coward and Webb, 1969; Stephens, 1972; Dixit, 1973; Schlichter, 1973). Most experiments attempting to demonstrate a nutritive role of these substances have utilized well fed animals presumably having high levels of metabolic substrates, although *a priori,* it is in the starved animal that one might expect a significant supplemental nutritional contribution by dissolved compounds.

There appears to be very little direct evidence supporting the hypothesis that dissolved amino acids (at environmentally realistic concentrations) are in fact an energy source for marine invertebrates. Stephens (1967) has pointed out that not all of the assimilated material is necessarily oxidized, but that it may also exert a sparing effect on a variety of metabolic pathways and on growth and reproductive processes. This implies that there need not necessarily be a net uptake of these compounds for them to be nutritionally significant. In the absence of data regarding the total flux of amino acids, an alternative course of investigation is to make qualitative and quantitative comparisons of biochemical, physiological and developmental processes among fed, starved and starved/amino acid-exposed animals. It is this approach that has been taken in the present study of *Aurelia aurita* polyps (scyphistomae) using dissolved glycine.

The uptake of dissolved free amino acids generally follows Michaelis-Menten kinetics, and while this does not necessarily imply that the uptake process is enzyme-mediated, the calculation of the kinetic constant K_t permits comparisons of the affinities of the amino acid uptake systems among different groups of animals. To date, the K_t values reported for marine invertebrates have been discussed almost exclusively in terms of the animals' adaptations to the concentrations of dissolved free amino acids in their respective habitats (Southward and Southward, 1972a, 1972b; Stephens, 1972), although an additional consideration has been demonstrated by Dixit (1973), who found ontogenetic differences in K_t for glycine uptake by a sea urchin. In view of the known influence of temperature on enzyme kinetics in poikilotherms (Hochachka and Somero, 1973), the investigation of the possibility of an analogous effect of temperature on the kinetics of amino acid uptake is clearly indicated.

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Somewhat surprisingly, temperature effects on uptake *per se* of dissolved organics by marine invertebrates remain generally unexamined, although Stephens (1962a) presented Q_{10} values for amino acid uptake by the maldanid polychaete *Clymenella torquata* and for glucose uptake by the coral *Fungia scutaria* (Stephens, 1962b). Likewise, whether or not the uptake process is subject to temperature acclimation is unknown. Considering the documentation of such effects on oxygen consumption and other parameters in marine invertebrates, it becomes obvious that a complete understanding of the significance of the uptake and utilization of dissolved amino acids must include an awareness of the thermal sensitivity of these processes.

Materials and Methods

Experimental animals

A clonal culture of *Aurelia aurita* scyphistomae from Corpus Christi, Texas was begun with a single animal from the culture isolated by Spangenberg (1964). The stock culture was maintained at room temperature (21°–24° C) in iodine-free, 30‰ artificial seawater to prevent strobilation. Polyps were fed *Artemia salina* nauplii twice weekly and food residues were removed from the culture bowls 1–3 days after each feeding. The water was changed monthly. A somewhat larger, genetically heterogeneous culture was maintained under conditions identical to those above, and polyps from this culture were used only in the determination of internal free amino acid pools.

Polyps of *A. aurita* from the York River, Virginia were provided by R. E. L. Black, College of William and Mary. These animals have a lower optimum salinity for growth and asexual reproduction than do the Texas scyphistomae, and were therefore maintained at 25‰. All other details of culture maintenance were as described above. These polyps were used only in the temperature acclimation studies described below, and to distinguish them from the Texas animals they are always specifically referred to as "Virginia" polyps or scyphistomae.

Temperature and starvation effects on glycine uptake

Texas polyps from the clonal culture were fed, brought to one of the acclimation temperatures (12°, 15°, 20°, 25°, 30° and 35° C) over periods ranging from 3–9 days, and again fed once the desired temperature had been attained. One group of polyps at each temperature was then fed twice weekly for 14 days, the last feeding being 2 days before the acclimated glycine uptake experiments were performed. A second group was deprived of food throughout the 14-day period.

At the end of the acclimation period, groups of 10 polyps of uniform size were removed from the culture dishes, adhering debris was removed with a pipet, and the animals were washed 4 times with Millipore filtered (0.45 μ pore size) artificial seawater at the acclimation temperature and salinity. Preliminary experiments in which polyps were preincubated for 21 hr in streptomycin sulfate (200 mg/l seawater) indicated that the above decontamination procedure was sufficient to eliminate any detectable effects of bacteria on total glycine uptake or distribution of radioactivity in the various fractions. Therefore, to minimize external variables, streptomycin was not used in the main body of experiments.

Decontaminated polyps were transferred to acid-rinsed, sterile test tubes in 0.5 ml of artificial seawater (30‰) at the acclimation temperature. To each tube was added 1.0 ml of water at the acclimation temperature and salinity containing [U-^{14}C] glycine (New England Nuclear), so that the final concentration of labeled glycine in the exposure medium was 0.80 μM. This concentration is ecologically realistic (*cf.* Webb and Wood, 1966; Siegel, 1967; Bohling, 1970, 1972; Clark, Jackson and North, 1972).

Preliminary experiments with Texas polyps at 20° C demonstrated that glycine uptake from a medium with an initial concentration of 0.80 μM is linear for at least 120 min; therefore, an exposure period of 1 hr was chosen for all subsequent experiments.

Polyps were exposed to the labeled medium in sealed test tubes for 1 hr at the acclimation temperature, quickly rinsed in 3 changes of artificial seawater and extracted overnight in 1.5 ml of 80% ethanol. The CO_2 was collected throughout the exposure period in 10% KOH on ground glass rods imbedded in the stoppers sealing the individual tubes; after removal of the animals, the exposure medium was acidified with 2 N HCl to drive off any remaining CO_2, which was also trapped in KOH. The extracted polyps were rinsed in 3 changes of clean ethanol and digested overnight in 0.5 ml of NCS Solubilizer (Amersham-Searle). Samples of ethanol extracts, EtOH insoluble material and KOH were prepared for counting as described in Shick (1973), and all samples were counted in Aquasol Universal L. C. S. Scintillator (New England Nuclear). Aliquots of the radioactive media were taken before and after the exposure period, and after acidification in the latter case. All samples were corrected for background and for quenching by use of both internal and external standards. Dry weights for each experimental group of animals were determined as in Shick (1973), with the addition that dried polyps were rinsed of salt before a second drying and reweighing.

The above exposure, CO_2 trapping, extraction, counting and weighing procedures were followed in all other experiments, with modifications described where appropriate. Unless otherwise noted, all experiments were performed in iodine-free artificial seawater of 30‰.

Acclimated rates of glycine uptake by fed (2-day) and starved (14-day) polyps were compared by Student's t test and by two-way analysis of variance (F test) performed on data obtained at temperatures common to both groups.

Temperature acclimation of glycine uptake rate

Groups of Texas polyps were maintained at 17° C (cold acclimated) and at 27° C (warm acclimated) for 2 weeks. Polyps were fed twice weekly, the last feeding being 2 days prior to the performance of uptake experiments at 17°, 22° and 27° C. An additional group was acclimated to 15° C, with uptake rates being determined at 15°, 22.5° and 30° C.

Groups of Virginia scyphistomae were gradually brought to 30‰ and maintained at 17° and 27° C under the same conditions as the Texas polyps. Other experimental procedures were also identical except that, due to their larger size, polyps were exposed to glycine in groups of 2 uniformly sized individuals at each temperature. Likewise, dry weight determinations on groups of 2 polyps were made as previously described.

Acutely determined rates of glycine uptake (*i.e.*, rates determined at temperatures other than those to which the animals were acclimated) were compared within each population using Student's t test and analysis of variance (F test).

Kinetics of glycine uptake

Groups of Texas polyps were fed twice weekly and acclimated to temperatures of 12°, 17°, 22°, 27° and 32° C for 14 days, at the end of which time they were exposed in groups of 5 to varying concentrations (0.12–101.36 μM; 3–5 replicates per concentration) of [U-^{14}C] glycine at their respective acclimation temperatures. A group of polyps maintained for 2 weeks at 20° C was placed in artificial seawater containing 1.5 μM potassium iodide and raised to 27° C to induce strobilation. Newly-liberated ephyrae were transferred to iodine-free water at 27° C and used in kinetic determinations at this temperature. The constants V_{max} (maximum uptake rate) and K_t (numerical equivalent of external glycine concentration at half-maximal uptake rate) were calculated from data at each temperature by means of least squares linear regression analyses performed on Eadie-Hofstee plots (v *vs.* v/[S]) of the Michaelis-Menten equation.

$^{14}CO_2$ production

Recently fed (2-day) and starved (14-day) Texas polyps were exposed to 0.80 μM [U-^{14}C] glycine for 1 hr as described above, and the CO_2 was collected throughout the exposure period. Other groups of polyps were thrice rinsed in clean artificial seawater following the exposure period and transferred to unlabeled medium, and CO_2 was collected after exposure plus incubation periods totalling 2, 3, 4, 6 and 8 hr.

In another series of experiments, groups of fed and starved Texas polyps were each exposed for 1 hr to either 1.28 μM [U-^{14}C] glycine or to 1.28 μM [2-^{14}C] glycine. Animals were incubated for 5 hr beyond the exposure period, during which time CO_2 was collected from all experimental groups. Samples were corrected for the specific activities of the two labeled glycine stock solutions in order to determine the relative contributions of the carboxyl and of the alpha carbon to $^{14}CO_2$ production by fed and starved polyps.

Effects of prolonged starvation

Seven groups of 20 clonal Texas scyphistomae were fed *ad libitum* during acclimation to 20° C. All groups were then decontaminated and placed separately in 25 ml of Millipore filtered artificial seawater and subjected to one of the following treatments. Two groups ("starved") were maintained at 20° C without food, the polyps being rinsed and sterile culture dishes and Millipore filtered water being changed daily for 56 days. The third ("starved/alanine-exposed") and fourth ("starved/glycine-exposed") groups were similarly treated, but in addition received 0.10 μM L-alanine or 0.80 μM glycine, respectively, in the daily renewed water; these concentrations approximate those found in estuarine and coastal waters. The fifth group ("starved/glucose-exposed") received 0.27 μM D-glucose, so that while this group had no external nitrogen source, it received

approximately the same amount of carbon as did the starved/glycine-exposed polyps. The sixth and seventh groups ("fed") were fed twice weekly during the entire 8-week period.

Buds produced asexually by all groups were counted daily for the duration of the experiment. All buds were removed as soon as they detached from the parent scyphistomae, since increased population density is known to affect the rate of asexual reproduction in *Aurelia* polyps (Coyne, 1973).

Bacterial counts of the water of each group except the fed were made on days 10, 36 and 56. One milliliter of culture water was added to 15 ml of medium containing 1.5 g Bacto-Peptone and 0.5 g Bacto-Agar (Difco Laboratories) per 100 ml of artificial seawater. Appropriate blanks were also prepared, and all plates were incubated at 20° C for 48 hr. While the plate method does not give an absolute count of bacterial cells per unit culture water (Wiebe, 1971), it provides a relative basis for comparisons among experimental cultures.

Following the 56-day maintenance period, polyps were placed in artificial seawater containing 1.5 μM potassium iodide and moved to a 27° C incubator to induce strobilation, and were examined daily for at least 14 days.

Oxygen consumption rates

Several groups of scyphistomae from the clonal culture were fed, brought to 20° C and again fed. One group was then fed twice weekly during maintenance

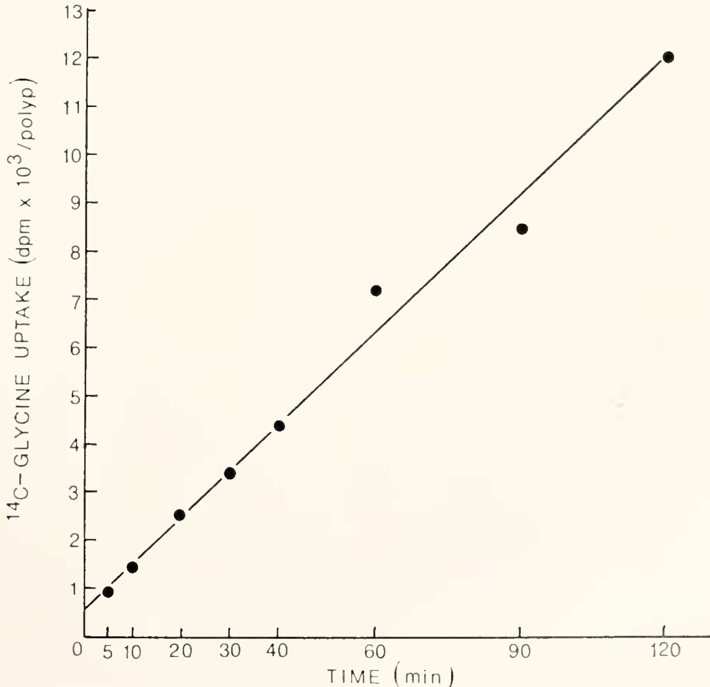

FIGURE 1. Uptake of [U-^{14}C] glycine from an initial concentration of 0.80 μM by groups of 10 Texas polyps as a function of exposure time.

at this temperature for 14 days, while the other 3 groups were deprived of food throughout this period. One group of starved polyps was exposed to 0.80 μM glycine during the last 20 hr of the food deprivation period. Another group of starved animals was exposed to the same concentration of glycine for 1 hr immediately prior to oxygen consumption determinations and a third group of starved polyps remained unexposed to exogenous glycine.

Groups of 50 fed polyps were decontaminated and placed in Millipore filtered artificial seawater at 20° ± 0.1° C in 53-ml vessels equipped with magnetic stirrers. Oxygen depletion in the sealed vessels was monitored continuously with a Yellow Springs Instrument Company Model 5450 polarographic electrode connected to a Model 54 oxygen meter. Following each experimental run, the oxygen consumption by the electrode alone was determined and subtracted from the experimental value. Identical procedures were followed in determinations of oxygen consumption rates in groups of 100–150 starved and starved/glycine-exposed polyps.

Polyps were dried at the completion of their respective runs. All oxygen consumption rates are expressed in terms of dry weight, and were compared using the Student-Newman-Keuls test.

Free amino acid pools

Groups of 100 fed and starved Texas polyps acclimated to 20° C and 30‰ were decontaminated and homogenized in 0.5 ml of absolute ethanol in an ice bath. The homogenate was centrifuged at 20,000 *g* for 20 min, the pellet dried and weighed, an aliquot of the supernatant removed for determination of total ninhydrin positive substances (NPS) according to the method of Clark (1964), and the remainder evaporated to dryness *in vacuo*. The residue was redissolved in 1% HCl and subjected to ion-exchange chromatography on a Beckman 120A amino acid analyzer using a 0.2 N citrate buffer at pH 3.25 and 4.28 on a 55-cm column of PA-28 resin at 55° C. Basic amino acids were not determined.

RESULTS

General

Glycine uptake by Texas polyps is linear for at least 120 min (Fig. 1). An exposure period of 1 hr was therefore chosen for all uptake experiments.

When considering all uptake experiments performed, irrespective of temperature or nutritive state, the range of dry weights of Texas polyps was 0.038–0.080 mg/polyp. In any one experiment, the dry weight of the fed animals never exceeded that of the starved by more than a factor of 2, and a weight correction for glycine uptake rate was not employed. Similarly, the weight range for Virginia polyps was 0.220–0.310 mg/polyp, and no weight correction has been made. Due to the considerable difference in weight between individuals of the 2 populations, however, their weight-specific glycine uptake rates may not be strictly comparable, and no such comparison has been attempted.

When the activities of the exposure media, ethanol soluble and insoluble materials, $^{14}CO_2$, and water and ethanol rinses were monitored, recovery of radioactivity ranged from 89–103% with a mean of 95.7%.

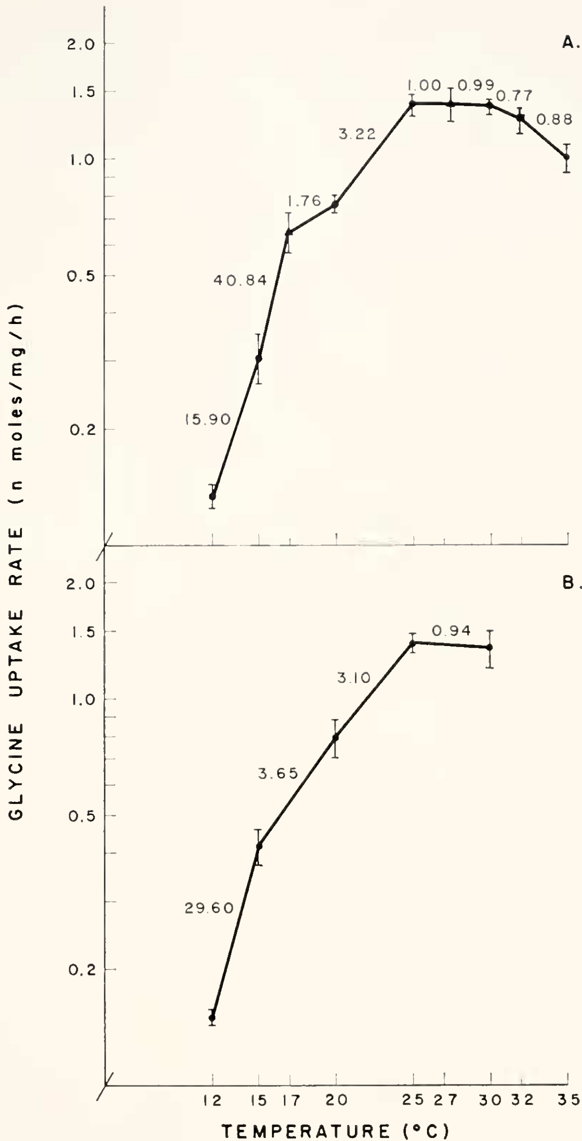

FIGURE 2. Acclimated [U-^{14}C] glycine uptake rates in fed (A) and starved (B) Texas polyps. Each point represents the mean of 5 groups of 10 animals ± SD. Included in (A) are values obtained in kinetic (squares) and temperature acclimation and kinetic (triangles) experiments. Q_{10} values given for each temperature interval.

Temperature and starvation effects on glycine uptake

Fed and starved polyps showed 100% survival at all acclimation temperatures, with the exception of 35° C where the starved animals exhibited 100%

TABLE I

Per cent of total radioactivity present as $^{14}CO_2$ in recently fed Texas polyps exposed to [U-^{14}C] glycine for 1 hr. Q_{10} values for each temperature interval given in parentheses.

Temperature										
12° C		15° C		20° C		25° C		30° C		35° C
0.02		0.05		0.31		0.48		0.66		0.37
	(21.20)		(38.44)		(2.40)		(1.89)		(0.31)	

mortality. While polyps at 10° C remained contracted, they gave no evidence of cyst formation, and did not survive prolonged exposure to this temperature. Feeding was observed at all acclimation temperatures.

Acclimated rates of glycine uptake for fed and starved polyps are shown in Figure 2. Additional comparable values obtained in kinetic and temperature acclimation experiments are plotted in this figure, although these data were not included in the statistical analyses. The acclimated uptake rate-temperature curve for fed animals indicates extreme thermal sensitivity of uptake in the intervals above the lower lethal limit, reduced sensitivity at intermediate temperatures, thermal insensitivity over the range of midsummer temperatures, and declining uptake as the incipient high lethal level is approached. The curve for starved polyps is virtually identical to that of the fed animals, although the stress of food deprivation did not permit survival at the highest temperature.

Student's t tests revealed significant differences in glycine uptake rates between fed and starved polyps only at 12° and 15° C ($P < 0.02$; $P < 0.01$, respectively). Although the gastrovascular cavities of most animals were empty of food residues 2 days after feeding, it is likely that a portion of the 12° C- and 15° C-acclimated polyps' weights was due to unassimilated and metabolically inactive materials, as evidenced by these polyps' longer retention of the color imparted by the carotenoids of the *Artemia* nauplii, leading to a lower apparent weight-specific uptake rate, and possibly accounting for the observed differences. Two-way analysis of variance revealed no significant effect of nutritive state ($P > 0.25$) or of nutritive state-temperature interaction ($P > 0.50$) on acclimated glycine uptake rates, while the effect of temperature was very highly significant ($P < 0.001$).

The per cent of radioactivity present as $^{14}CO_2$ (Table I), which may be taken as an index of metabolic activity, shows a pattern of temperature sensitivity similar to that of glycine uptake. For convenience of comparison, Q_{10} values are given for each temperature interval, but it must be noted that $^{14}CO_2$ production is expressed in terms of relative, not absolute, rates. The considerable increase in the rate of glycine uptake in the 15° to 20° C interval is paralleled by a sixfold increase in relative $^{14}CO_2$ production over this range of temperature. Like glycine uptake, $^{14}CO_2$ production declines above 30° C, a manifestation of the effects of thermal stress.

Temperature acclimation of glycine uptake rate

Acutely determined dissolved glycine uptake rates in cold acclimated (15°, 17° C) and warm acclimated (27° C) Texas scyphistomae, and in similarly acclimated Virginia polyps (omitting the 15° C-acclimated group) are shown in Figure 3. It is immediately apparent that both sets of curves exhibit the comparatively rare pattern of reverse translation (Prosser, 1964), wherein the curve

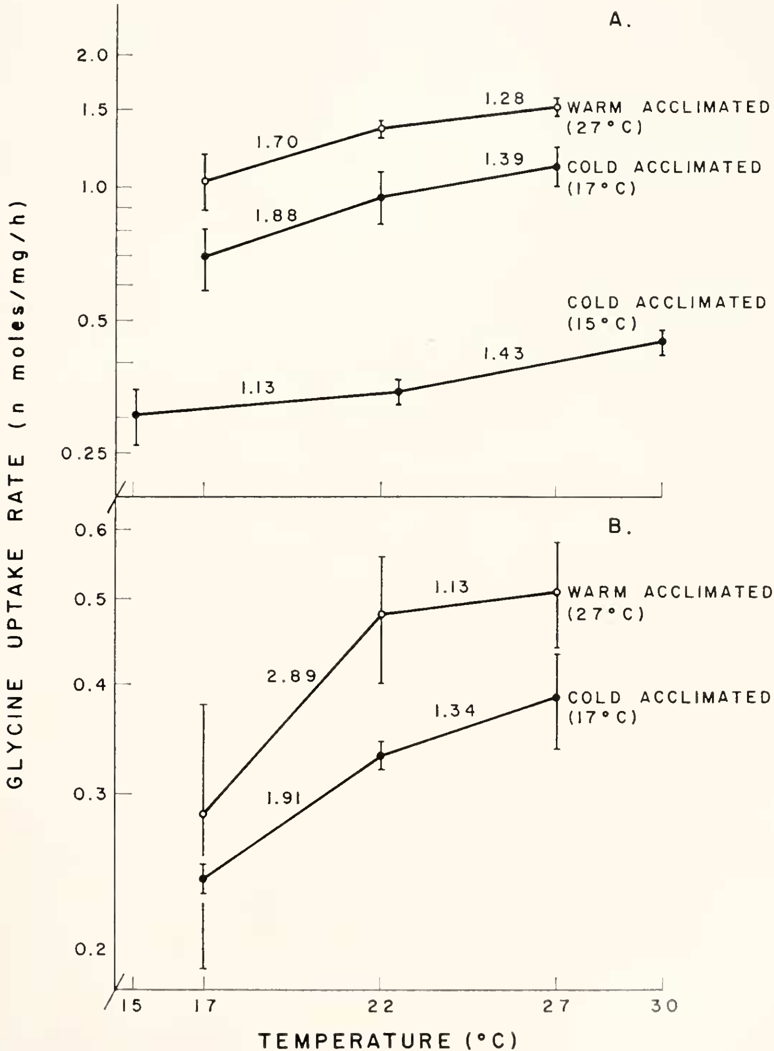

FIGURE 3. Acute [U-^{14}C] glycine uptake rates in Corpus Christi, Texas polyps (A), and in York River, Virginia polyps (B). Each point in (A) represents the mean of 5 groups of 10 animals ± SD; each point in (B) represents the mean of 5 groups of 2 animals ± SD. Q_{10} values given for each temperature interval.

of the warm acclimated animals is displaced upward and to the left of that of the cold acclimated individuals. Student's t tests performed on acute rates in 17° C- and 27° C-acclimated animals within both populations revealed significant differences ($P < 0.025$) in all comparisons except the Virginia polyps at 17° C (see Fig. 3B). Divergence between the curves of the 17° C- and 27° C-acclimated polyps in both populations, as determined by analysis of variance, is significant (Texas: $P < 0.005$; Virginia: $P < 0.01$).

The acute measurements were made after the acclimated rates of uptake had been determined. The latter experiments had indicated that 17° C was the threshold below which glycine uptake rates in Texas polyps exhibit extreme thermal sensitivity (Fig. 2A), and accordingly, 17° C was chosen as the cold maintenance temperature. Acute determinations performed on Texas polyps maintained at 15° C, below the aforementioned threshold, did in fact reveal a considerable depression in uptake rates relative to the 17° C-acclimated animals (Fig. 3A). The ecological significance of the 17° C threshold is not obvious.

Kinetics of glycine uptake

The constants K_t and V_{max} at each acclimation temperature are given in Table II. Values of both constants are directly related to acclimation temperature. The correlation coefficients indicate a high degree of linearity in regression analyses of Eadie-Hofstee plots of data at all temperatures, and suggest that the diffusion component of uptake is not large. The latter also follows from the large concentration gradient against which glycine is taken up by the polyps. The possibility of glycine transport in *Aurelia* polyps by carrier-mediated exchange diffusion has not been examined, although Wong (1971) and Stephens (1972) have shown that this is not the case in other marine invertebrates. When all acclimation temperatures and concentrations of labeled glycine in the media are considered, the amount of radioactivity recovered as $^{14}CO_2$ and ethanol insoluble material ranges from 0.7–4.3% of the total uptake; thus, the effects of temperature on K_t and V_{max} are genuine and not artifacts resulting from the metabolic removal of labeled glycine from the soluble pool.

The K_t and V_{max} for glycine uptake by ephyrae at 27° C are also given in Table II. While there is little difference between polyps and ephyrae in the

TABLE II

Effects of temperature on K_t and V_{max} of glycine uptake by Texas polyps. Values determined from linear regression analyses of Eadie-Hofstee plots of data.

Temperature (°C)	K_t ($\times 10^{-5}$ M)	V_{max} (n moles mg dry weight^{-1} h^{-1})	Correlation coefficient
12	0.79	1.61	−0.984
17	0.89	6.49	−0.967
22	1.23	20.16	−0.971
27	2.35	39.32	−0.947
27 (ephyrae)	2.49	93.51	−0.925
32	3.89	64.13	−0.963

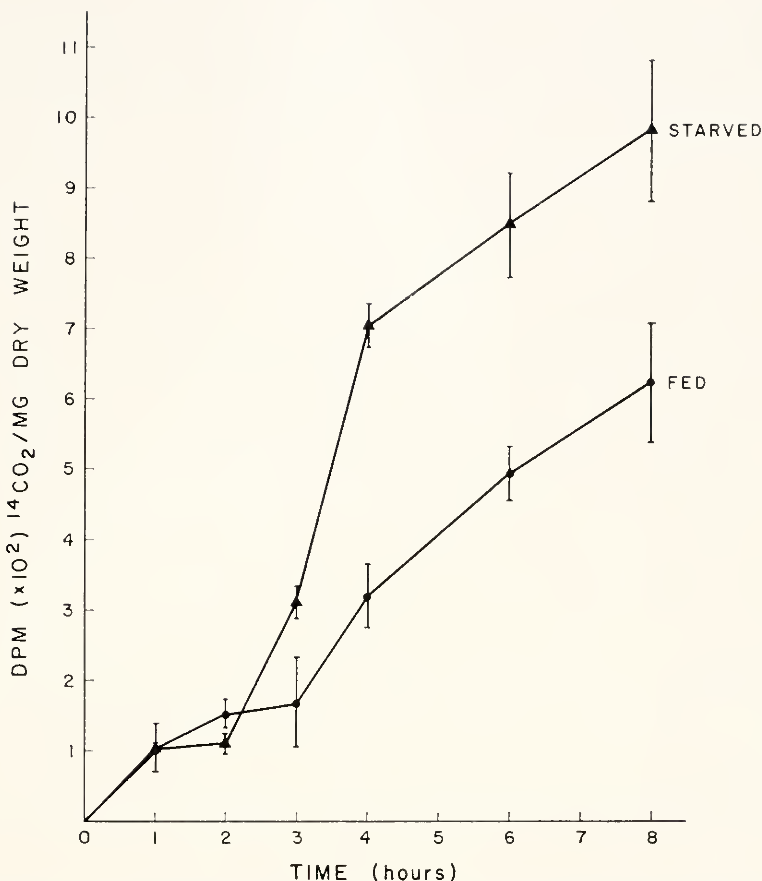

FIGURE 4. Time course of $^{14}CO_2$ production by fed (circles) and starved (triangles) Texas polyps following a 1-hr exposure to [U-^{14}C] glycine. Each point represents the mean of 4 groups of 10 animals ± SD.

values of K_t, ephyrae have a somewhat higher weight-specific V_{max}. The possible significance of these observations is discussed below.

$^{14}CO_2$ production

The time course of $^{14}CO_2$ production by fed and starved polyps exposed to 0.80 μM [U-^{14}C] glycine is shown in Figure 4. There is no apparent difference in the rate of $^{14}CO_2$ production between the 2 groups during the first 2 hr, following which there is a large increase in the rate of its production by the starved polyps.

The total $^{14}CO_2$ production by the starved polyps then stabilizes at a level almost double that of the fed animals, similar to the results of the longer-term incubations reported by Shick (1973). Conversely, the percentage incorporation of labeled glycine into ethanol insoluble materials during the 1-hr exposure period decreases after 2 weeks of starvation (Table III).

TABLE III

Per cent of total radioactivity present as ethanol insoluble material in fed and starved Texas polyps exposed to [U-^{14}C] glycine for 1 hr.

	Temperature					
	12° C	15° C	20° C	25° C	30° C	35° C
Fed	1.40	2.18	2.59	2.57	2.03	3.28
Starved	1.05	0.76	0.69	1.89	1.99	—

It was found that 82% of the $^{14}CO_2$ produced by fed animals was derived from the carboxyl carbon, 18% being derived from the alpha carbon (Table IV). In starved animals these values are 64% and 36%, respectively, indicating a more complete breakdown of the glycine molecules taken up from solution by these polyps. Corrected values for labeled ethanol soluble and insoluble materials are also given in Table IV; chi-square analyses revealed no significant differences between fed and starved polyps in derivation of labeled carbon in these fractions.

Effects of prolonged starvation

Quantitative effects of prolonged starvation on strobilation and budding, with and without concomitant exposure to dissolved organic matter (DOM), are given in Table V, as are bacterial counts of the water from the groups deprived of solid food. Survival during 56 days of starvation was excellent, the figure of 97.5% in the "starved" groups being due to loss of a single damaged polyp during the first week.

Production of buds by the fed polyps continued throughout the entire period, but ceased after 14–15 days in all other groups. Likewise, there is little difference among the latter groups in total number of buds produced.

The minimum time to strobilation initiation (in this study, referring to the first evidence of either constriction or flattening of the polyp) after exposure to

TABLE IV

Uptake of radioactive glycine (Gly) and distribution of label among various fractions corrected for specific activities of the two radioactive glycine stock solutions, and expressed as dpm/mg dry weight ± SD, where n = 3 groups of 10 Texas polyps at both nutritive states and glycine sources.

Nutritive state	Fraction	Radioactive source		Derivation of ^{14}C in fractions (carboxyl-^{14}C; α-^{14}C)
		[U-^{14}C] Gly	[2-^{14}C] Gly	
Fed	CO_2	648 ± 83	115 ± 23	(82%; 18%)
Fed	EtOH soluble	242,034 ± 9816	263,447 ± 18,215	(48%; 52%)
Fed	EtOH insoluble	18,901 ± 2199	19,400 ± 743	(50%; 50%)
Starved	CO_2	4112 ± 366	1465 ± 213	(64%; 36%)
Starved	EtOH soluble	329,675 ± 23,952	350,659 ± 17,240	(49%; 51%)
Starved	EtOH insoluble	17,753 ± 2191	20,250 ± 1188	(47%; 53%)

TABLE V

Results of prolonged starvation (56 days at 20° C), with and without concomitant exposure to dissolved organic compounds, on strobilation and budding in Texas polyps. See text for discussion of qualitative differences among groups.

	Fed*	Starved*	Starved/ alanine	Starved/ glycine	Starved/ glucose
% Survival	100, 100 (100)	95, 100 (97.5)	100	100	100
Total buds produced by 20 polyps	217, 263	17, 21	16	19	17
Time to 1st observed strobilation initiation (days)	3, 3	5, 5	3	3	3
% Strobilating	100, 100 (100)	20, 25 (22.5)	100	100	100
Ephyrae produced per polyp	3.8, 3.5	1.0, 1.0	1.6	1.2	1.4
Abnormal ephyrae/total ephyrae	3/77, 4/69	2/4, 3/5	5/32	3/24	15/27
Bacterial colonies/ml culture water (average of determinations on days 10, 36 and 56)	—	7.4, 6.0	3.4	2.8	4.5

* Data for 2 groups of 20 polyps.

iodide and temperature increase also varied among the treatment groups. The shorter time was observed in the fed and the starved/DOM-exposed groups, and the longer in the starved.

The most dramatic and informative difference among the treatment groups is the per cent of animals strobilating. All polyps in the fed and in the starved/DOM-exposed groups produced ephyrae, while the response was reduced to 22.5% in the starved animals. Among the groups deprived of solid food, ephyra production was exclusively via monodisk strobilation in the starved polyps, while at least some of the starved/DOM-exposed polyps produced 2 ephyrae. Typical polydisk strobilae were observed in the fed groups.

A high percentage of the ephyrae produced by starved and starved/glucose-exposed polyps exhibited severe developmental and morphological anomalies. Such abnormalities were seen less frequently among fed and starved/amino acid-exposed groups. An ephyra was considered to be abnormal if it had other than the normal 8 bifurcated marginal lobes, or if it was grossly misshapen. The significance of these observations is considered below.

Zobell and Feltham (1938) presented evidence of bacteria serving as a food source for a number of marine invertebrates, and DiSalvo (1971) and Sorokin (1973) have extended this observation to corals. Percival (1923) demonstrated that *Aurelia* scyphistomae ingest carmine particles transported to the mouth in ciliated tracts on the body surface. These lines of evidence, taken together, suggest that scyphistomae could feed on bacteria present in the water and such a mechanism, if employed by the polyps, could overshadow the nutritive role of dissolved organic compounds suggested by the results of the above experiments. However, the daily changing of the culture dishes and the Millipore filtered water,

the daily rinsing of the polyps, and the 20° C maintenance temperature kept bacterial growth to comparable minima in the starved cultures (Table V). It does not seem likely that the minimal growth affected the experimental results.

Oxygen consumption rates

Oxygen consumption rates in fed, starved and starved/glycine-exposed polyps are given in Figure 5. The rates are mean values for oxygen consumption rates over a 5-hr period by 4 groups of polyps at each experimental condition. Attempts to correlate oxygen consumption with increases in $^{14}CO_2$ production in the 2–4 hr interval after glycine exposure (Fig. 4) proved inconclusive. While starved polyps exposed to glycine for 1 hr exhibited no consistent increase in oxygen consumption in the 2–4 hr interval during the continuous measurements, the rates were highly variable both among groups and within a given group over the time course of the determinations, perhaps reflecting metabolic instability during the activation of catabolic pathways.

The rates in the 3 groups deprived of solid food are significantly lower than the rate in fed polyps ($P < 0.001$ in all cases). The rate in starved/glycine-exposed (20 hr) polyps is significantly greater ($P < 0.05$) than that in the starved scyphistomae. The rate in starved/glycine-exposed (1 hr) polyps does not differ significantly ($P > 0.05$) from that in starved polyps.

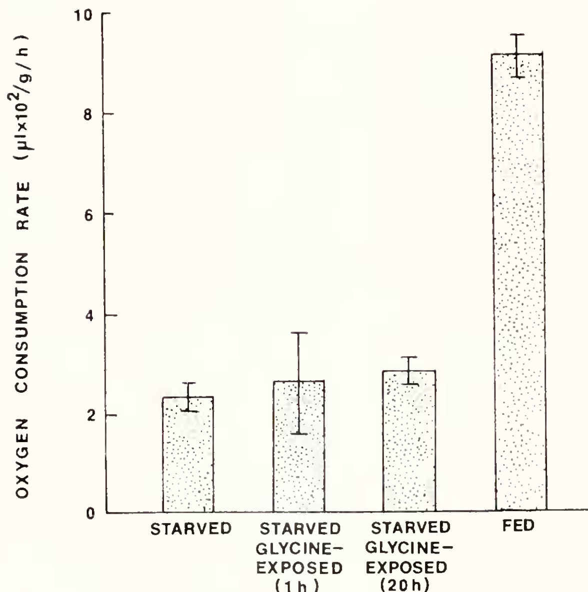

FIGURE 5. Oxygen consumption rates in 4 groups of 100–150 starved, starved/glycine-exposed (1 hr), and starved/glycine-exposed (20 hr) Texas polyps, and in 4 groups of 50 fed polyps. Values are means ± SD.

Free amino acid pools

Absolute amounts of acidic and neutral free amino acids (FAA) in pools of *Aurelia aurita* scyphistomae from Corpus Christi, Texas are presented elsewhere (Shick, 1974). The data show a general similarity to those for *Aurelia* from the York River, Virginia given by Webb, Schimpf and Olmon (1972), in that glycine and taurine predominate and that β-alanine is present.

Reliable wet tissue weights were not obtained, and values are necessarily expressed in terms of amount of amino acid per unit dry weight. While the concentrations of most FAA decline during 2 weeks of starvation, those of glycine, taurine and β-alanine increase from 89 to 154, 29 to 49, and 23 to 48 nmole/mg, respectively. The total FAA content (excluding undetermined basic amino acids) increases from 306 to 343 nmole/mg, although the actual *concentrations* may be more similar due to increased tissue hydration in the starved polyps.

Discussion

This study provides direct evidence of a nutritional role of dissolved amino acids at environmentally realistic concentrations in starved *Aurelia aurita* scyphistomae. In addition, substantial data regarding temperature effects on the uptake of glycine, and on the kinetics of uptake, are presented.

The temperature sensitivity of glycine uptake by Texas *Aurelia* polyps (Fig. 2) is similar to that of other rate processes in warm-temperate zone scyphozoans, for example, the pulsation rate-temperature curve for *Aurelia* ephyrae given in Mangum, Oakes and Schick (1972), the pulsation R-T curve for *Chrysaora quinquecirrha* medusae in Gatz, Kennedy and Mihursky (1973), and the curves for *Aurelia* and *Cassiopea xamachana* medusae presented by Mayer (1914). It must be noted that the pulsation rates in *Aurelia* ephyrae and medusae, and in *Cassiopea* medusae, were acutely determined; only in the studies on *Chrysaora* medusae and in the present investigation of *Aurelia* polyps were the animals acclimated to the experimental temperatures.

Of considerable interest is the pattern of inverse temperature compensation of glycine uptake shown by the Texas polyps (Fig. 3A). Such an observation is not unprecedented, having also been noted in the uptake of solutes by bacteria and yeasts (Christophersen, 1967), and in the accumulation of ninhydrin positive substances by *Mya arenaria* (DuPaul and Webb, 1970). The latter work, however, involved apparently endogenously-derived materials accumulated by adductor muscle in response to increased salinity.

While Virginia polyps exhibit positive thermal acclimation of metabolic rate (Mangum *et al.*, 1972), they also show a pattern of inverse compensation of glycine uptake (Fig. 3B), similar to that of the Texas polyps, and the inverse compensation is therefore apparently not a latitudinal phenomenon. The inverse compensation is not necessarily maladaptive. On the contrary, since seasonal acclimation of oxygen consumption and other metabolic processes is less than perfect in scyphozoan polyps and medusae (*i.e.*, Q_{10} values > 1.0 [Mangum *et al.*, 1972; Gatz *et al.*, 1973]), it is advantageous for the warm acclimated animals to have an enhanced rate of metabolite transport.

The increasing affinity of the uptake system for glycine, indicated by the de-

creasing K_t values, as the acclimation temperature is lowered (Table II) would seem to be an example of seasonal "positive thermal modulation" as discussed by Hochochka and Somero (1973) for enzyme kinetics. If one accepts the premise that the uptake of glycine is mediated by a membrane transport protein or "enzyme" (for reviews, see: Stein, 1967; Pardee, 1968), then the decreasing K_t values with decreasing acclimation temperature may indeed be analogous to the phenomenon of positive thermal modulation described for enzyme kinetics.

From the results of the temperature acclimation experiments already discussed, however, it is obvious that the entire process of glycine uptake is not strictly analogous to that of enzyme activity. The reasons for the seeming discrepancy between inverse temperature compensation of glycine uptake and positive thermal modulation of the affinity of the uptake system are likely to be numerous, but essentially rest in the fact that the experiments were performed on intact animals. While the transport system exhibits increasing affinity for glycine as the temperature is lowered, the total influx of glycine is differently affected, being greater in the warm acclimated polyps, as evidenced by the inverse temperature compensation and by the large temperature effect on V_{max} (Table II). This may result from a reduced number of available transport sites, due to changes in membrane lipid composition, or from a diminished turnover of glycine by the carrier, in the cold acclimated polyps.

Previous investigators have discussed variation of K_t values for amino acid uptake by marine invertebrates almost exclusively with reference to the animals' adaptations to the environmental free amino acid concentrations to which they are normally exposed (Southward and Southward, 1972a, 1972b; Stephens, 1972; Dixit, 1973). The K_t value for glycine uptake by *Aurelia* ephyrae is essentially the same as that of the polyps at the same temperature (Table II). If the environmental FAA concentration is the principal determining factor for the affinity of the amino acid uptake system, it would appear that the polyp, which is epifaunal on a variety of firm substrates, and the ephyra, an actively swimming plankter, are adapted to the same environmental glycine concentration. The microstratification of dissolved organic matter is poorly understood; however, the difference in DFAA concentration is likely to be greater between the interstitial water and that at the sediment surface than among the latter and different levels in the water column. Analyses of water from different microhabitats are certainly necessary to clarify this point.

While the above consideration is no doubt important and perhaps ultimately the prime determinant, the effect of environmental temperature on K_t values has not been considered heretofore. The present study has demonstrated a significant temperature effect on K_t values for glycine uptake by *Aurelia* polyps. The values at the lower acclimation temperatures are within the range of K_t values for amino acids given by Southward and Southward (1970, 1972a, 1972b) for deep sea pogonophorans. At the highest temperature they extend within the range of K_t values for amino acid uptake by a variety of marine and estuarine invertebrates summarized by Stephens (1967) and Dixit (1973). The K_t value for glycine uptake by *Chrysaora quinquecirrha* scyphistomae is 19 μM (K. L. Webb, personal communication), at the middle of the range of values reported for *Aurelia* polyps in the present investigation. The K_t values summarized by Stephens and by

Dixit, and that reported by Webb, were determined at or near room temperature; the experiments by the Southwards were performed at 4°–6° C, and the K_t's which they reported would presumably increase at higher (although unnatural for their specimens) temperatures. It becomes evident that intra- and interspecific comparisons of affinities of amino acid uptake systems based solely on known environmental DFAA concentrations may permit only an incomplete understanding of the implications of the process, since a temperature effect on K_t is an additional modifying factor in eurythermal invertebrates.

The results of several lines of experimentation demonstrate that dissolved glycine, at the environmentally realistic concentration of 0.80 μM, is a supplemental nutritional source for starved *Aurelia aurita* scyphistomae.

While polyps of *Aurelia* are known to be resistant to prolonged starvation (Spangenberg, 1967), the degree of nutritive preparation has a demonstrably profound effect on strobilation (Thiel, 1962; Spangenberg, 1967; Russell, 1970). The results summarized in Table V indicate that starvation of Texas polyps for 56 days markedly reduces the incidence of strobilation. Of greater import to the present discussion is the observation that this diminution of the number of animals strobilating can be abolished by exposing the polyps to environmental concentrations of glycine or alanine during the period of food deprivation. Additional results in Table V, as well as qualitative differences in strobilation (developmental anomalies, meristic variation in ephyrae, *etc.*) between starved and starved/amino acid-exposed polyps, further demonstrate that dissolved amino acids are indeed a supplemental nutritional source for these animals during 56 days of starvation in the laboratory.

It therefore seemed particularly worthwhile to determine whether the importance of these molecules is as a source of reduced carbon for energy-yielding metabolism (*i.e.,* as a "supplemental energy source"), or of nitrogen for amino and nucleic acids, or whether a combination of factors is involved. Accordingly, subsequent experiments were performed in which polyps were given dissolved glucose during the 8-week food deprivation period. The concentration was adjusted so that polyps in this group had approximately the same amount of carbon available to them as did the starved/glycine-exposed polyps, the principal difference being the presence or absence of exogenous nitrogen.

Most importantly, it was found that the diminution of the strobilation response in starved animals can also be abolished by their exposure to dissolved glucose. Thus, both nitrogenous and non-nitrogenous substrates may exert a sparing effect on endogenous polyp materials, which are converted to ephyra materials during metamorphosis.

Serious developmental anomalies and meristic variation occur with a high frequency in starved polyps and in those receiving glucose, but to a much lesser extent in starved/amino acid-exposed and in fed animals (Table V). That exogenous nitrogen sources are necessary for normal metamorphosis and development is therefore obvious, especially when one recalls that the experimental animals are genetically identical. The demonstrated 41–260% increases in DNA per polyp and probable continuous synthesis of RNA and protein during strobilation (Black, 1972) further emphasize the importance of nitrogenous materials during

this period, and may give some insight as to at what levels the dissolved organic nitrogen sources in the present experiment exert their effects.

The results of this entire line of experimentation indicate that the polyp-to-ephyra transformation is of fundamental importance in *Aurelia aurita*, and once induced and initiated, proceeds to its conclusion even under severe nutritive stress. These results may also help to explain a variety of well known observations on *Aurelia*. Developmental anomalies and meristic variation have been studied in *Aurelia* medusae and ephyrae at least since 1837 (see discussions in Berrill, 1949; Thiel, 1959; and Russell, 1970). While the possibility remains that much of the observed morphological variation is due to genetic differences among individuals and to undetermined environmental factors, the present investigation, using clonal polyps, has demonstrated that nutritional conditions play an extensive role in producing such variations. The production of both normal and abnormal (specifically, those having more than 8 lobes) ephyrae by the same well fed strobila, also discussed by Berrill (1949) remains unexplained, although it is apparently a fairly common occurrence.

Exposure of starved polyps to dissolved organic compounds has no effect on the process of budding. All groups of starved polyps, whether or not they were exposed to these compounds, ceased budding after 14–15 days of food deprivation. This cessation is reflected in the decreased percentage incorporation of labeled glycine into ethanol insoluble materials by starved animals (Table III, and Shick, 1973). It would not be advantageous for a population of relatively sessile polyps to increase its size, and hence its demand for food, during periods of food scarcity. If dissolved amino acids and other organic molecules have a supplemental nutritional role or sparing effect on developmental processes, this role is more likely to be manifested in the process of strobilation, which provides a means for animals to leave the local population (*i.e.*, by swimming away as ephyrae), and assures dispersal of the sexual stage of the life cycle.

Aurelia ephyrae have a higher weight-specific V_{max} for glycine uptake than do the polyps (Table II). It is unknown whether this is due to the smaller size of the former, to their proportionally greater surface area, or to an increase in the number of carrier sites or turnover of glycine. Whichever is the case, the importance of a greater uptake rate to the ephyra, a planktonic larval stage with limited nutrient reserves, yet with a much higher activity level and energy demand than the sessile polyp (Mangum *et al.*, 1972), seems obvious.

Oxygen consumption by marine invertebrates is known to decline during food deprivation (Roberts, 1957; Vernberg, 1959; Thompson and Bayne, 1972; Bayne, 1973a, 1973b; Newell, 1973; Wallace, 1973; and others), although the time course over which this occurs is extremely variable among the organisms studied. Oxygen consumption decreases to 25.9% of the value in fed polyps in *Aurelia* scyphistomae starved for 14 days (Fig. 5).

That oxygen consumption increases significantly in starved polyps following exposure to environmental concentrations of dissolved glycine (Fig. 5) may further indicate an energy-providing role of dissolved glycine. While the total glycine flux in fed and starved polyps remains undetermined, such an activation of catabolic pathways does not seem likely if there is a net loss of material. The possibility that the increase is a reflection of the initiation of the feeding response

by glycine (Loeb and Blanquet, 1973) cannot be discounted, although the persistence of a higher rate of oxygen consumption after termination of 20 hr of glycine exposure would seem to argue against it.

Qualitative and quantitative analyses of the FAA pools of fed and starved polyps reveal that while the concentrations of most free amino acids decline during 2 weeks of food deprivation, there is little change in the internal FAA concentration. This concentration is maintained largely by increases in glycine, taurine and β-alanine. These amino acids are the principal constituents of the pools of the starved scyphistomae, and are known to be important in the total osmotic concentration of the tissues of other marine invertebrates. Their increased concentrations in starved polyps may be a compensatory mechanism to maintain the internal concentration of osmotically active substances during the utilization of other amino acids in the pool. The presence of β-alanine in the Texas scyphistomae is also significant from the standpoint of biochemical divergence among geographically separated *Aurelia aurita* populations (Webb *et al.*, 1972; Morales-Alamo and Haven, 1974; Shick, 1974).

Since the internal free glycine concentration actually increases in polyps after 14 days of food deprivation, and since these animals take up the same amount of labeled glycine per unit body weight as do fed animals (Fig. 2), then the increase in $^{14}CO_2$ production by starved scyphistomae (Fig. 4) does in fact demonstrate an enhanced catabolism of glycine by these animals, as tentatively suggested by Shick (1973). The enhancement of glycine catabolism by starved polyps is due at least in part to the more complete breakdown of the glycine molecule by these animals, as indicated by the increased (doubled) appearance of alpha-carbon label in $^{14}CO_2$ (Table IV). The lag in $^{14}CO_2$ production by starved polyps, followed by its rapid appearance (Fig. 4), may be a further indication of the activation of catabolic pathways as suggested by the oxygen consumption determinations. Since the absolute and percentage glycine concentration rises considerably in starved polyps, and since the uptake of exogenous glycine would tend to accentuate this imbalance, the basis for the increased glycine catabolism may be the offsetting of such an imbalance.

The pathways of glycine catabolism in *Aurelia* polyps remain unclear. Glycine catabolism does not normally proceed via the citric acid cycle (for discussion, see Shick, 1973). In addition, unpublished personal observations suggest that while the occurrence of the glyoxylate cycle in *Aurelia*, as proposed by Raum (1970), cannot be discounted, it appears to be of little quantitative significance in glycine catabolism.

Despite the above facts, a large amount of $^{14}CO_2$ is produced from the alpha carbon of labeled glycine by *Aurelia* scyphistomae. It is now known that the oxidative glycine cleavage system of ammonotelic animals can produce CO_2 from the alpha carbon (Kikuchi, 1973) and this might account for the observations in the present study. The predominance of carboxyl-carbon radioactivity in the $^{14}CO_2$ produced by fed polyps (Table IV) indicates that the labeled glycine is broken down primarily via decarboxylation, perhaps in a glycine cleavage system, with the alpha carbon being channelled to the one-carbon pool for biosynthetic processes. The increased production of CO_2 from the alpha carbon by

starved scyphistomae would provide additional reduced pyridine nucleotide for increased energy production in these polyps.

Assuming that glycine is catabolized via the glycine cleavage system and not via the citric acid cycle or glyoxylate cycle, then the complete breakdown of 1 mole of glycine would produce 1 mole each of $NADH_2$ and $NADPH_2$ (Kikuchi, 1973), which would require 1 mole of O_2 for reoxidation. The glycine uptake rate at 20° C from an ambient glycine concentration of 0.80 μM could then provide sufficient glycine to support approximately 6% of the observed oxygen consumption rate in starved/glycine-exposed polyps. Glycine, while calorically poor relative to other amino acids, is generally the most concentrated amino acid dissolved in seawater (Webb and Wood, 1966; Siegel, 1967; Hobbie, Crawford and Webb, 1968; Bohling, 1970; Andrews and Williams, 1971; Clark *et al.,* 1972). This fact, coupled with its increased oxidation during starvation, may enhance its importance as a supplemental energy source for marine invertebrates.

The long-term starvation experiments (Table V) also demonstrated the importance of glycine as a source of nitrogen. The glycine uptake rate in starved polyps at 20° C and 0.80 μM glycine could provide about 0.26 μg N per starved polyp (average dry weight 35 μg) during the 56-day starvation period. Assuming that roughly 1–3% of the dry weight of semaeostome scyphozoans is nitrogen (Vinogradov, 1953, pages 198–199), this means that starved/glycine-exposed polyps take up nitrogen equivalent to 25–74% of their total body nitrogen during the starvation period.

The above calculations do not take into consideration the amount of glycine released by the polyps through leakage or other means. Such information, while difficult to obtain accurately, is certainly necessary if the significance of glycine uptake is to be stated quantitatively.

It has become apparent that a full understanding of the significance of the uptake of dissolved amino acids must take into account a number of factors. While environmental temperature, salinity and DFAA concentration determine the magnitude and dynamics of the uptake of these compounds by the animal in question, nutritive state has a direct bearing on the allocation of these materials between catabolic and anabolic pathways. Comparisons among fed, starved and starved/amino acid-exposed *Aurelia aurita* scyphistomae have shown that dissolved amino acids are important in the alleviation of nutritive stress in these organisms, this importance being manifested in biochemical, physiological and developmental processes.

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Summary

1. The temperature sensitivity of glycine uptake by *Aurelia aurita* scyphistomae from Corpus Christi, Texas is similar to that of other rate processes in warm-temperate zone scyphozoans.

2. Both Texas polyps and those from the York River, Virginia show inverse temperature compensation of glycine uptake; the phenomenon is therefore apparently not latitudinally based.

3. The values of K_t and V_{max} for glycine uptake are directly related to temperature between 12° and 32° C. The increasing affinity of the glycine uptake system with decreasing temperature may be analogous to "positive thermal modulation" of enzyme-substrate affinity in poikilotherms.

4. The fivefold increase in K_t between 12° and 32° C indicates that environmental temperature is an important consideration in intra- and interspecific comparisons of the affinities of amino acid uptake systems in marine invertebrates.

5. Eight weeks of food deprivation at 20° C result in a 77.5% reduction in the number of polyps strobilating in response to temperature increase and exposure to iodide. This effect can be abolished by exposing starved polyps to environmental concentrations of glycine or alanine during the starvation period.

6. Exposure of starved polyps to dissolved glucose during the 8-week period also overrides the diminution of the strobilation response. However, starved and starved/glucose-exposed polyps produce a higher percentage of abnormal ephyrae than do fed and starved/amino acid-exposed polyps, emphasizing the importance of dissolved amino acids as nitrogen sources.

7. All starved polyps, whether or not they are exposed to dissolved organic compounds, cease budding after 14–15 days of food deprivation.

8. Oxygen consumption declines to 25.9% of the value in fed polyps during 2 weeks of food deprivation. Exposure of starved polyps to dissolved glycine produces an increase in this parameter.

9. There is no effect of 2 weeks of food deprivation on glycine uptake by polyps. However, starvation does produce an enhanced rate of glycine catabolism, due in part to the increased production of CO_2 from the alpha carbon of the molecule. The predominance of glycine among amino acids dissolved in seawater, and its increased oxidation during starvation, may enhance its importance as a supplemental energy source for marine invertebrates.

10. The internal pool concentrations of most free amino acids decline during 2 weeks of food deprivation; the total FAA concentration of the pools is little affected, largely due to compensatory increases in glycine, taurine and β-alanine.

LITERATURE CITED

ANDREWS, P., AND P. J. LEB. WILLIAMS, 1971. Heterotrophic utilization of dissolved organic compounds in the sea. III. Measurements of the oxidation rates and concentrations of glucose and amino acids in sea water. *J. Mar. Biol. Ass. U.K.*, **51**: 111–125.

BAYNE, B., 1973a. Aspects of the metabolism of *Mytilus edulis* during starvation. *Netherlands J. Sea Res.*, **7**: 399–410.

BAYNE, B. L., 1973b. Physiological changes in *Mytilus edulis* L. induced by temperature and nutritive stress. *J. Mar. Biol. Ass. U.K.*, **53**: 39–58.

BERRILL, N. J., 1949. Developmental analysis of scyphomedusae. *Biol. Rev.*, **24**: 393–410.

Black, R. E., 1972. Nucleic acid and protein levels in strobilating polyps of *Chrysaora quinquecirrha* and *Aurelia aurita*. *Mar. Biol.,* **16**: 134–137.

Bohling, H., 1970. Untersuchungen über freie gelöste Aminosäuren in Meerwasser. *Mar. Biol.,* **6**: 213–225.

Bohling, H., 1972. Gelöste Aminosäuren in Oberflächenwasser der Nordsee bei Helgoland: Konzentrationsveränderungen im Sommer 1970. *Mar. Biol.,* **16**: 281–289.

Christophersen, J., 1967. Adaptive temperature responses of microorganisms. Pages 327–348 in C. L. Prosser, Ed., *Molecular Mechanisms of Temperature Adaptation.* American Association for the Advancement of Science, Washington, D. C.

Clark, M. E., 1964. Biochemical studies on the coelomic fluid of *Nephtys hombergi* (Polychaeta: Nephtyidae), with observations on changes during different physiological states. *Biol. Bull.,* **127**: 63–84.

Clark, M. E., G. A. Jackson and W. J. North, 1972. Dissolved free amino acids in southern California coastal waters. *Limnol. Oceanogr.,* **17**: 749–758.

Coast and Geodetic Survey, 1968. *Surface Water Temperature and Density, Atlantic Coast, North and South America.* Coast and Geodetic Survey Publication 31–1, Washington, D. C., 102 pp.

Coyne, J. A., 1973. An investigation of the dynamics of population growth and control in scyphistomae of the scyphozoan *Aurelia aurita. Chesapeake Sci.,* **14**: 55–58.

DiSalvo, L. H., 1971. Ingestion and assimilation of bacteria by two scleractinian coral species. Pages 129–136 in H. M. Lenhoff, L. Muscatine and L. V. Davis, Eds., *Experimental Coelenterate Biology.* University of Hawaii Press, Honolulu.

Dixit, D. B., 1973. Uptake of amino acids and development in the sea urchin, *Strongylocentrotus purpuratus. Ph.D. dissertation, University of California, Irvine,* 117 pp.

DuPaul, W. D., and K. L. Webb, 1970. The effect of temperature on salinity-induced changes in the free amino acid pool of *Mya arenaria. Comp. Biochem. Physiol.,* **32**: 785–801.

Gatz, A. J., Jr., V. S. Kennedy and J. A. Mihursky, 1973. Effects of temperature on activity and mortality of the scyphozoan medusa, *Chrysaora quinquecirrha. Chesapeake Sci.,* **14**: 171–180.

Hobbie, J. E., C. C. Crawford and K. L. Webb, 1968. Amino acid flux in an estuary. *Science,* **159**: 1463–1464.

Hochachka, P. W., and G. N. Somero, 1973. *Strategies of Biochemical Adaptation.* Saunders, Philadelphia, 358 pp.

Johannes, R. E., S. J. Coward and K. L. Webb, 1969. Are dissolved amino acids an energy source for marine invertebrates? *Comp. Biochem. Physiol.,* **29**: 283–288.

Kikuchi, G., 1973. The glycine cleavage system: Composition, reaction mechanism, and physiological significance. *Mol. Cell. Biochem.,* **1**: 169–187.

Loeb, M. J., and R. S. Blanquet, 1973. Feeding behavior in polyps of the Chesapeake Bay sea nettle, *Chrysaora quinquecirrha* (Desor, 1848). *Biol. Bull.,* **145**: 150–158.

Mangum, C. P., M. J. Oakes and J. M. Shick, 1972. Rate-temperature responses in scyphozoan medusae and polyps. *Mar. Biol.,* **15**: 298–303.

Mayer, A. G., 1914. The effects of temperature upon tropical marine animals. *Pap. Tortugas Lab.,* 6: 1–24.

Morales-Alamo, R., and D. S. Haven, 1974. Atypical mouth shape of polyps of the jellyfish, *Aurelia aurita,* from Chesapeake Bay, Delaware Bay, and Gulf of Mexico. *Chesapeake Sci.,* **15**: 22–29.

Newell, R. C., 1973. Factors affecting the respiration of intertidal invertebrates. *Amer. Zool.,* **13**: 513–528.

Pardee, A. B., 1968. Membrane transport proteins. *Science,* **162**: 632–637.

Percival, E., 1923. On the strobilization of *Aurelia. Quart. J. Microscop. Sci.,* **67**: 85–100.

Prosser, C. L., 1964. Perspectives of adaptation: Theoretical aspects. Pages 11–25 in D. B. Dill, E. F. Adolph and C. G. Wilber, Eds., *Handbook of Physiology.* Section 4. American Physiological Society, Washington, D. C.

Raum, W. J., 1970. Glycine metabolism in the scyphistoma of *Aurelia aurita. M.A. thesis, Bowling Green State University,* 21 pp.

Roberts, J. L., 1957. Thermal acclimation of metabolism in the crab *Pachygrapsus crassipes*

Randall. I. The influence of body size, starvation, and molting. *Physiol. Zoöl.*, **30**: 232–242.

Russell, F. S., 1970. *The Medusae of the British Isles, Vol. 2, Pelagic Scyphozoa with a Supplement to the First Volume on Hydromedusae.* The University Press, Cambridge, 284 pp.

Schlichter, D., 1973. Ernährungsphysiologische und ökologische Aspekte der Aufnahme in Meerwasser gelöster Aminosäuren durch *Anemonia sulcata* (Coelenterata, Anthozoa). *Oecologia*, **11**: 315–350.

Shick, J. M., 1973. Effects of salinity and starvation on the uptake and utilization of dissolved glycine by *Aurelia aurita* polyps. *Biol. Bull.*, **144**: 172–179.

Shick, J. M., 1974. Uptake and utilization of dissolved glycine by *Aurelia aurita* scyphistomae: Temperature effects on the uptake process; nutritional role of dissolved amino acids. *Ph.D. dissertation, Texas A&M University*, 87 pp.

Siegel, A., 1967. A new approach to the concentration of trace organics in seawater. Pages 235–256 in T. A. Olson and F. J. Burgess, Eds., *Pollution and Marine Ecology.* Wiley-Interscience, New York.

Sorokin, Yu. I., 1973. On the feeding of some scleractinian corals with bacteria and dissolved organic matter. *Limnol. Oceanogr.*, **18**: 380–385.

Southward, A. J., and E. C. Southward, 1970. Observations on the role of dissolved organic compounds in the nutrition of benthic invertebrates. Experiments on three species of Pogonophora. *Sarsia*, **45**: 69–96.

Southward, A. J., and E. C. Southward, 1972a. Observations on the role of dissolved organic compounds in the nutrition of benthic invertebrates. II. Uptake by other animals living in the same habitat as pogonophores, and by some littoral Polychaeta. *Sarsia*, **48**: 61–70.

Southward, A. J., and E. C. Southward, 1972b. Observations on the role of dissolved organic compounds in the nutrition of benthic invertebrates. III. Uptake in relation to organic content of the water. *Sarsia*, **50**: 29–46.

Spangenberg, D. B., 1964. New observations on *Aurelia*. *Trans. Amer. Microscop. Soc.*, **83**: 448–455.

Spangenberg, D. B., 1967. Iodine induction of metamorphosis in *Aurelia*. *J. Exp. Zool.*, **165**: 441–450.

Stein, W. D., 1967. *The Movement of Molecules across Cell Membranes.* Academic Press, New York, 369 pp.

Stephens, G. C., 1962a. Uptake of amino acids by the bamboo worm, *Clymenella torquata*. *Biol. Bull.*, **123**: 512.

Stephens, G. C., 1962b. Uptake of organic material by aquatic invertebrates. I. Uptake of glucose by the solitary coral, *Fungia scutaria*. *Biol. Bull.*, **123**: 648–659.

Stephens, G. C., 1967. Dissolved organic material as a nutritional source for marine and estuarine invertebrates. Pages 367–373 in G. H. Lauff, Ed., *Estuaries.* American Association for the Advancement of Science, Washington, D. C.

Stephens, G. C., 1972. Amino acid accumulation and assimilation in marine organisms. Pages 155–184 in J. W. Campbell and L. Goldstein, Eds., *Symposium on Nitrogen Metabolism and the Environment.* Academic Press, New York.

Thiel, Hj., 1962. Untersuchungen über die Strobilisation von *Aurelia aurita* Lam. an einer Population der Kieler Förde. *Kieler Meeresforsch.*, **18**: 198–230.

Thiel, M. E., 1959. Scyphomedusae. Semaeostomeae, Physiologie. *Bronn's Kl. Ordn. Tierreichs 2, Abt. II*, **2**(6): 849–1072.

Thompson, R. J., and B. L. Bayne, 1972. Active metabolism associated with feeding in the mussel *Mytilus edulis* L. *J. Exp. Mar. Biol. Ecol.*, **9**: 111–124.

Vernberg, F. J., 1959. Studies on the physiological variation between tropical and temperate zone fiddler crabs of the genus *Uca*. II. Oxygen consumption of whole organisms. *Biol. Bull.*, **117**: 163–184.

Vinogradov, A. P., 1953. *The Elementary Chemical Composition of Marine Organisms.* Memoir 2, Sears Foundation for Marine Research, Yale University, New Haven, 647 pp.

Wallace, J. C., 1973. Feeding, starvation and metabolic rate in the shore crab *Carcinus maenas*. *Mar. Biol.*, **20**: 277–281.

Webb, K. L., A. L. Schimpf and J. Olmon, 1972. Free amino acid composition of scyphozoan polyps of *Aurelia aurita, Chrysaora quinquecirrha* and *Cyanea capillata* at various salinities. *Comp. Biochem. Physiol.*, **43B**: 653–663.

Webb, K. L., and L. Wood, 1966. Improved techniques for analysis of free amino acids in seawater. Pages 440–444 in *Automation in Analytical Chemistry*, Technicon Symposia, Vol. 1. Mediad, White Plains, New York.

Wiebe, W. J., 1971. Perspectives in microbial ecology. Pages 484–497 in E. P. Odum, *Fundamentals of Ecology*. [3rd Ed.] Saunders, Philadelphia.

Wong, L., 1971. Uptake of amino acids by marine annelids. I. Mechanisms of uptake and rate of loss. II. Regulatory control and effect on free amino acid pool. *Ph.D. dissertation, University of California, Irvine*, 130 pp.

Zobell, C. E., and C. B. Feltham, 1938. Bacteria as food for certain marine invertebrates. *J. Mar. Res.*, **1**: 312–327.